REMARKS

Applicants have amended the specification and claims to more particularly define the invention taking into consideration the outstanding Official Action. Applicants submit that the 112 rejections of claims 24-43 have been obviated by Applicants' amendment. Applicants further note that the 102(b) rejections of claims 24-30, 32-33, 35-39, 41-42 and 44 have been withdrawn. Applicants have canceled claims 24-57 from the present application and have replaced them with new claims 58-91. Claims 58 to 91 correspond generally to the canceled claims but have been rewritten to obviate the outstanding rejections and are fully supported by the specification as originally filed. Applicants submit that the claims now present in the application are fully supported by the specification as originally filed and no new matter is introduced.

The objection to the specification because the suggested subject headings are absent has been obviated in view of the amendments to the specification to provide these headings. Accordingly, it is most respectfully requested that this objection be withdrawn.

The objection to claims 24, 26, 35-36, 38, 44, 45, 52-53 and 57 because of the informalities set forth on page 4 of the outstanding Official Action has been carefully considered but is most respectfully traversed in view of the cancellation of the claims from the present application and the newly added claims now present in the application.

Applicants submit that the term "SAH" is now shown in parenthesis in new claim 58 following the first occasion of the un-abbreviated term in this claim. Accordingly, it is requested that this objection be withdrawn.

Further, the phrase "a first enzyme being" is deleted from all claims, such that the corresponding enzyme is now specified as "the homocysteine converting enzyme SAH hydrolase". The term "second" is also deleted, so that the previous second enzyme is now termed simply "an enzyme capable of...". The term "secondary antibody" is now used consistently throughout the claims to correspond to "primary antibody" and avoid any confusion over its identity. The Examiner's helpful suggestions are appreciated. Accordingly, it is most respectfully requested that these objections be withdrawn.

The rejection of claims 24-57 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement has been carefully considered but is most respectfully traversed in view of the cancellation of the rejected claims and the newly added claims and following comments.

The Examiner objects to the amended wording introduced to describe the polyhapten in the previous amendment. This is on the basis that the word "comprising" is boarder than was originally disclosed in the application. Applicants believe that no change in scope was in fact made, since the original wording simply required that SAH hapten moieties were present. The original term "having SAH as hapten moieties" does not exclude the possibility that further hapten moieties are present. Nonetheless, in order to avoid any issue in this respect, and to more specifically define the polyhapten in relation to inventive step, as discussed below, the wording objected to is not present in the newly added claims, and thus the objection is believed moot.

The Examiner considers the definition of "antibody" provided in the specification as filed and believes that the specification fails to provide suitable evidence that the inventors were in full possession of the invention to extent of this definition at the time the application was filed. In particular, the Examiner rejects the claims as lacking written support for oligopeptide or oligonucleotide binders having affinity for the polyhapten complex. Although the inventors believe that methods for generating and screening arrays of specific binding molecules, such as aptamers, are well know in the art, the enclosed description amendment deletes the terms "oligopeptides" and "oligonucleotides" to expedite prosecution of the application.

The Examiner objects to the inclusion of the term "antibody fragments" in the definition of antibodies within the specification. This is on the basis that:

> "In the instant case, the claims are drawn to methods of using any conceivable antibody fragment..... one skilled in the art would not envisage possession of methods of using all antibody fragments to capture antigen, since only those *that retain antigen-binding variable regions* would bind to antigen" (original emphasis).

In the claims, however, "all antibody fragments" have never been claimed. What is claimed is "a primary antibody capable of binding to said polyhapten" and "a secondary antibody capable of binding to said complex". The Examiner is quite correct

in that where these antibodies are in fact antibody fragments, the specific binding regions must be (at least to a large extent) retained, but those are the fragments which are claimed, since those are the fragments which meet the requirements of the claim. It has always been the case that an applicant is entitled to a reasonable generalisation of the invention which he demonstrates, and the Examiner is content that full length antibodies and certain (Fab) fragments are adequately described. Other fragments, however, are generated from full length antibodies by the same enzymatic digestion method used to generate Fab fragments, and the method has been common in the art for many years as would be appreciated by one of ordinary skill in the art. Enclosed is an excerpt from the standard cell biology text-book Alberts et al., *Molecular Biology of the Cell* (1989) as illustrative of this. The Examiner's attention is particularly drawn to Figure 18-16.

A skilled worker can generate differing antibody fragments by digestion of full length enzymes by other peptidases. He can then test the binding of these by the same routine methods which the specification teaches for selecting the original antibodies. He is therefore put in full possession of the information required to make and test many antibody fragments and ensure that these fulfil the binding requirements laid out in the claim without undue experimentation. Since only those antibody fragments having the recited specific binding properties are claimed, it is moot whether the inventors were able to use, for example, an antibody fragment consisting of a heavy chain only, as proposed by the Examiner, since this` would not fulfil these requirements and thus is not claimed. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 24-35 and 45-52 under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps has been carefully considered but is most respectfully traversed in view of the cancellation to the claims and the newly added claims and following comments.

The Examiner rejects all method claims as incomplete for lacking an essential step in the claimed method, the method being to an assay for homocysteine and there being no final step relating the result to this component. Clearly such a relationship was

intended by the inventors at the time of application, since the claim could not otherwise be to "a method for assaying homocysteine". This is also demonstrated in the examples of the application as filed, where the final result is the derivation of the homocysteine concentration in the sample. In the independent method claims as herewith amended, the further step of relating the photometrically detected complex to the homocysteine content of said sample is thus now explicitly recited.

In amending the claims, the level of one of ordinary skill in the art has been taken into consideration and it is believe that such a skilled person would find the claims definite. Accordingly, it is most respectfully requested that this rejection be withdrawn.

Applicants most respectfully submit that all of the claims now present in the application are in full compliance with 35 USC 112 and clearly patentable over the references of record.

The rejection of claims 24-26, 29-30, 32-33, 36-39, 41-42, 45, 48, 49-51 and 53-56 under 35 U.S.C. 103(a) as being unpatentable over Frantzen et al. in view of Zuk et al. has been carefully considered but is most respectfully traversed in view of the cancellation to the claims and the newly added claims and following comments.

Applicants wish to direct the Examiner's attention to the basic requirements of a prima facie case of obviousness as set forth in the MPEP § 2143. This section states that to establish a prima facie case of obviousness, three basic criteria first must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Section 2143.03 states that all claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." In re Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). If an

independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

Applicants also most respectfully direct the Examiner's attention to MPEP § 2144.08 (page 2100-114) wherein it is stated that Office personnel should consider all rebuttal argument and evidence presented by applicant and the citation of In re Soni for error in not considering evidence presented in the specification.

Frantzen provides for a highly effective assay for Hcy which is optimised for use with an automated analyser. Importantly, however, this assay method is different in type to that of the present invention. In the present claims, a soluble polyhapten is employed (it must be soluble or in stable dispersion because it is part of the first stable aqueous reagent mixture). When the primary antibody is added, this cross-links the polyhapten units, causing them to precipitate, thereby generating a signal which can be detected photometrically. When homocysteine is present in the mixture, this is converted (with adenosine) by SAH-hydrolase to SAH, and the SAH then competes for binding to the primary antibody, thereby reducing the cross-linking and lowering the amount of precipitate. Since all of the necessary reagents are in two or three solutions, the assay can be carried out quickly and easily in any suitable vessel.

In order to emphasise the discrete nature of the polyhapten as a component of the first reagent mixture, rather than as a modification of the surface of a vessel, the polyhapten is now limited to "having at least one hapten moiety per 100kD of its molecular weight and having a molecular weight in the range 500 kD to 3 MD", based upon lines 6 to 10 of page 8 of the application as filed.

In contrast to the above method, the method of Frantzen includes no polyhapten according to the meaning used in the present invention. SAH is used in Frantzen, but this is not in a soluble form. Instead, the SAH is attached directly to the surface of a micro-titre plate. The effect of homocysteine in the sample is similar, in that this produces SAH which competes for binding to an anti-SAH antibody, but rather than generating a signal directly, Frantzen requires the addition of further antibodies, enzymes and substrates to generate the signal. In all, six separate aqueous reagents are used in the method of Frantzen, and in addition, one of the reagents (the SAH) must be pre-immobilised on the surface of the plates, meaning that only specially

prepared plates can be used in the assay. The figure at the top of page 313 excellently illustrates the many steps and reagents required in the method of Frantzen. It is important to appreciate that precipitation is not part of the assay in Frantzen, since the SAH haptens are already bound to the micro-titre plate, and a species already immobilised to a surface cannot precipitate. Frantzen thus requires further binding and enzymatic reactions to generate a signal.

It is the stated goal of the Frantzen work to provide a fully automated homocysteine assay method avoiding chromatography and radioisotopes (last 3 lines of the first column, page 311). One of the most important issues when designing such an assay is ease of reagent handling, since automatic analysers typically handle only a small number of separate reagents, and the greater the total number of reagent addition steps, the more machine time a single assay with require.

It is notable that Frantzen, the lead author of this citation is the inventor in the present case, and so it is clear that he was aware of the value of reducing complexity and reducing the number of reagents in an automated assay method for homocysteine. The Frantzen document <u>teaches directly away form the present method and reagents as would be appreciated by one of ordinary skill in the art to which the invention pertains</u>, however, since it suggests that even a homocysteine assay which has been optimised for automation requires multiple aqueous reagents. It furthermore teaches a method involving an immobilised SAH hapten pre-attached to the surface of the reaction vessel. This teaches away from precipitation type techniques and directly away from the possibility of including the binder for the anti-SAH antibody in one of the aqueous reagents. The Examiner believes that Frantzen teaches the same reagents as the present claims, but Applicants submit that this is not the case; the immobilised SAH of Frantzen could never be the polyhapten stable in aqueous solution which is required by the present claims.

In order to render a claim obvious, the cited art must disclose or teach towards every aspect of the claim. In the present case, Frantzen teaches away from the possibility of a small number of aqueous reagents in a homocysteine assay, since its optimised assay employs many more. It furthermore teaches directly away from the use of a soluble polyhapten by requiring the use of pre-treated micro-titre assay plates.

These features are not taught in Zuk, and thus the combination of these documents fails to teach key features of the present claims.

Turning to the contents of Zuk, this document relates specifically to one particular and unusual type of immunoassay. In this, an immuno-complex is formed which serves to protect a labelled ligand from a subsequent step which would change or eliminate the label (see summary of the invention section). This is supposedly suitable down to 1 μg/ml, which would make the method only just theoretically capable of measuring homocysteine at normal concentrations in blood (around 10 μM, or 1.35 μg / ml), even if the sample could be prepared without any dilution.

The Examiner particularly cites lines 22 to 68 of column 22 as teaching the advantage of kits, and that it is desirable to combine the reagents. This teaching, however, is specific to the unique assay method of Zuk, with particular reference to the "loading factor" for giving optimal sensitivity, and the fact that Fab fractions (in the Zuk method) can be combined with their haptens without undue problems. Obviously, this is not a generally applicable teaching.

Even to the extent that the teaching supposedly present in Zuk is available, this provides only a desire to reduce the number of separate reagents in a known assay method. The inventors of the presently claimed invention were required to contribute a great deal more than this obvious desideratum in order to complete the present invention. In particular, there are a very many possible assay formats which could be employed for measuring homocysteine, even when the basic enzymatic conversion with SAH-hydrolase is decided upon. There are three components (two reactants and one product) of the SAH-hydrolase reaction, these may then be measured by further enzymatic conversion, or by direct binding, such a binder may be an antibody or non-functional enzyme, and the binding may then be measure by competition, by displacement, with a labelled analogue, with a binder to the bound complex, etc, and the label may be of many sorts (enzymatic, fluorescent, colorimetric etc). Once an assay format is found, it is then necessary to consider how many groups of incompatible reagents, are present, and how many separate steps are required. It is of no value generating a stable solution containing some of the reagents for step 1, and some reagents which must not be added until step 6, even if they are stable together

in storage. The exact assay format is therefore embodied in the reagent mixtures, because those reagents which are mixed must always be added concomitantly.

Of all of these options, the present inventors have established that by using a soluble poly-hapten precipitation assay format, and by separating the required components of the assay in claimed way, only two or three separate reagents can be provided which can be added to give all the necessary steps and components of the assay in the correct order. At most, Zuk is a general teaching of the desirability of fewer reagents. Since Frantzen, addressing the same problem shortly before does not achieve anything like so elegant a solution, it is of no value simply to say that one should use fewer mixtures; The method of Frantzen requires six steps, and thus six mixtures at least will be required, or the assay would be carried out in fewer steps. It is the combination of exact assay format and specific combinations and separations of reagents which allows the remarkably efficient method of the present claims, and only specific teaching to these could give a skilled worker any expectation that he could successfully create a homocysteine assay using only two or three stable reagent mixtures. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 24-30, 32-33, 36-39, 41-42, 45-51 and 53-56 under 35 U.S.C. 103(a) as being unpatentable over Sundrehagen et al. in view of Zuk. has been carefully considered but is most respectfully traversed in view of the cancellation to the claims and the newly added claims and following comments.

The Examiner rejects a similar selection of claims over the combination of Sundrehagen and Zuk, but the sections of Sundrehagen cited serve only the emphasise the number of possible assay formats, methods and reagents which a skilled worker must investigate before he can begin to reduce the number of stable reagents required to the claimed number. The Examiner notes that polyhaptens are mentioned, but this is only one of a considerable number of possible formats. Sundrehagen does not suggest that this format is amenable to reducing the number of assay steps to a minimum, nor that the reagents required in this method would need to be added in such an order that those which are incompatible may be segregated into separate mixtures but still added at the correct stage of the assay.

As indicated above, the most which is shown by Zuk is an obvious desire to reduce reagents in an abstract way. Zuk does not address homocysteine, nor does he suggest how one might analyse possible assay methods in order to select that one which may be most amenable to combining its reagents into single mixtures without violating the order in which they must be applied. Without such specific teaching, there can be no possible expectation of success if a skilled worker sought to create a homocysteine assay having two or three stable reagents, and that it is possible is only evident with full hindsight knowledge of the present invention. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 30-31 and 39-40 under 35 U.S.C. 103(a) as being unpatentable over Frantzen et al. in view of Zuk et al., or alternatively over Sundrahagen et al. in view of Zuk et al. as applied to claims 24 and 36 above, and further in view of either Karl et al. or Lin et al. has been carefully considered but is most respectfully traversed in view of the cancellation to the claims and the newly added claims and following comments.

Applicants most respectfully submit that nothing in this combination, however, at least brings in an essential teaching which is missing from Frantzen, Sundrehagen and Zuk, as discussed above, and without that teaching no claim of the present set can be obvious. The Examiner's comments in this section are thus moot in view of the inventive nature of the independent claims. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 34 and 43 under 35 U.S.C. 103(a) as being unpatentable over Frantzen et al. in view of Zuk et al., or alternatively over Sundrahagen et al. in view of Zuk et al. as applied to claims 33 and 42 above, and further in view of Yanaihara et al. has been carefully considered but is most respectfully traversed in view of the cancellation to the claims and the newly added claims and following comments.

However, any consideration of these dependent claims is believed moot since the essential nature of the independent claims is not taught towards in any citation. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 35, 44, 52 and 57 under 35 U.S.C. 103(a) as being unpatentable over Frantzen et al. in view of Zuk et al., or alternatively over

Sundrahagen et al. in view of Zuk et al. as applied to claims 24-25 and 36-37 above, and further in view of Hideo et al. or de Steenwinkel et al. has been carefully considered but is most respectfully traversed in view of the cancellation to the claims and the newly added claims and following comments.

Applicants most respectfully submit, however, the presence or absence of this additional restriction in the prior art is believed moot since the essential nature of the independent claims is not taught towards in any citation. Accordingly, it is most respectfully requested that this rejection be withdrawn.

In view of the above comments and further amendments to the claims favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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weight. In this section we shall describe the five classes of antibodies found in higher vertebrates, each of which mediates a characteristic biological response following antigen binding.

The Antigen-specific Receptors on B Cells Are Antibody Molecules[12]

As predicted by the clonal selection theory, all antibody molecules made by an individual B cell have the same antigen-binding site. The first antibodies made by a newly formed B cell are not secreted; instead, they are inserted into the plasma membrane, where they serve as receptors for antigen. Each B cell has approximately 10^5 such antibody molecules in its plasma membrane.

When antigen binds to the antibody molecules on the surface of a virgin or a memory B cell, it usually initiates a complicated series of events culminating in cell proliferation and maturation to produce either memory cells or active (antibody-secreting) cells (see p. 1047). The active cells make large amounts of soluble (rather than membrane-bound) antibody with the same antigen-binding site as the cell-surface antibody and secrete it into the blood. Active B cells can begin secreting antibody while they are still small lymphocytes, but the end stage of the maturation pathway is the large plasma cell (see Figure 18–4B), which secretes antibodies at the rate of about 2000 molecules per second. Plasma cells seem to have committed so much of their protein-synthesizing machinery to making antibody that they are incapable of further growth and division and die after several days.

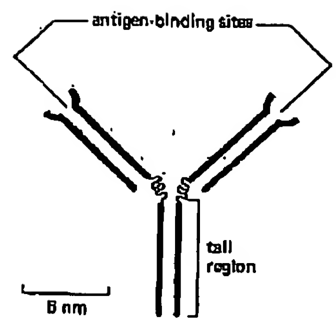

Figure 18–12 A simple representation of an antibody molecule.

B Cells Can Be Stimulated to Make Antibodies in a Culture Dish[13]

Two advances in the 1960s revolutionized research on B cells. The first was the development of the **hemolytic plaque assay**, which made it possible to identify and count individual active B cells secreting antibody against a specific antigen. In the simplest form of this assay, lymphocytes (commonly from the spleen) are taken from animals that have been immunized with sheep red blood cells (SRBCs). They are then embedded in agar together with an excess of SRBCs so that the dish contains a "lawn" of immobilized SRBCs with occasional lymphocytes in it. Under these conditions the cells are unable to move, but any anti-SRBC antibody secreted by a B cell will diffuse outward and coat all SRBCs in the vicinity of the secreting cell. Once the SRBCs are coated with antibody, they can be killed by adding complement (see p. 1031). In this way the presence of each antibody-secreting cell is indicated by the presence of a clear spot, or *plaque*, in the opaque layer of SRBCs. The same assay can be used to count cells making antibody to other antigens, such as proteins or polysaccharides, if these antigens are coupled to the surface of the SRBC.

The second important advance was the demonstration that B lymphocytes can be induced to make antibody by exposing them to antigen in culture, where cell interactions can be manipulated and the environment controlled. This led to the discovery that both T lymphocytes and specialized *antigen-presenting cells* (see p. 1045) are required for most antigens to stimulate B lymphocytes to secrete antibodies; the cell-cell interactions involved will be described later (see p. 1047).

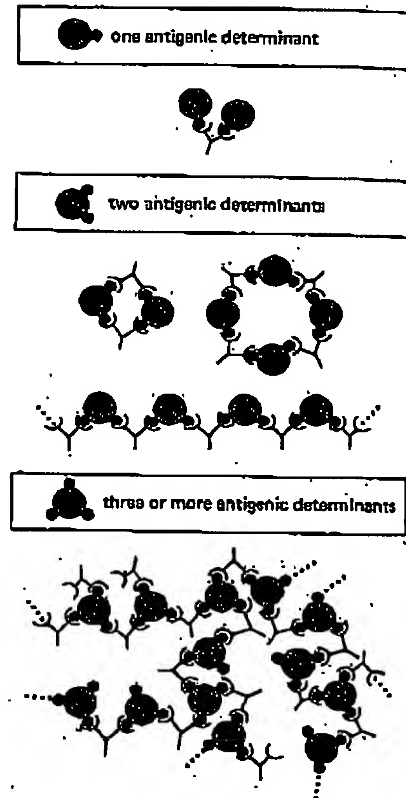

Figure 18–13 Because antibodies have two identical antigen-binding sites, they can cross-link antigens. The types of antibody-antigen complexes that form depend on the number of antigenic determinants on the antigen. Here a single species of antibody (a monoclonal antibody) is shown binding to antigens containing one, two, or three copies of a single type of antigenic determinant. Antigens with two antigenic determinants can form small cyclic complexes or linear chains with antibody, while antigens with three or more antigenic determinants can form large three-dimensional lattices that readily precipitate.

Antibodies Have Two Identical Antigen-binding Sites[11]

The simplest antibodies are Y-shaped molecules with two identical **antigen-binding sites**, one at the tip of each arm of the Y (Figure 18–12). Because of their two antigen-binding sites, they are said to be *bivalent*. Such antibody molecules can cross-link antigen molecules into a large lattice as long as each antigen molecule has three or more antigenic determinants (Figure 18–13). Once it reaches a certain size, such a lattice precipitates out of solution. This tendency of large immune complexes to precipitate can be used to detect the presence of antibodies and antigens. The efficiency of antigen binding and cross-linking is greatly increased by a flexible *hinge region* in antibodies, which allows the distance between the two antigen-binding sites to vary (Figure 18–14).

The protective effect of antibodies is not due simply to their ability to bind antigen. They engage in a variety of activities that are mediated by the tail of the Y-shaped molecule. This part of the molecule determines what will happen to the antigen once it is bound. Because of the way immunoglobulins are synthesized (see p. 1029), antibodies with the same antigen-binding sites can have any one of several different tail regions, each of which confers on the antibody different functional properties, such as the ability to activate complement (see p. 1032) or to bind to phagocytic cells (see p. 1015).

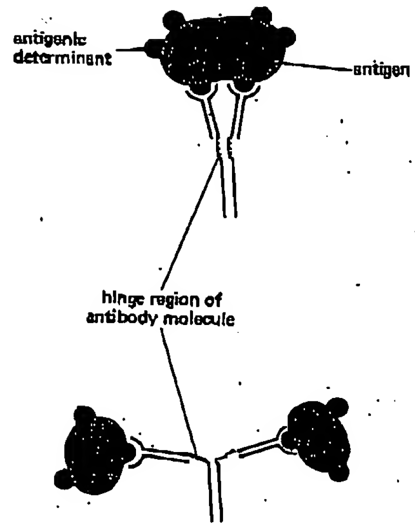

Figure 18–14 The hinge region of an antibody molecule improves the efficiency of antigen binding and cross-linking.

An Antibody Molecule Is Composed of Two Identical Light Chains and Two Identical Heavy Chains[14]

The basic structural unit of an antibody molecule consists of four polypeptide chains, two identical **light (L) chains** (each containing about 220 amino acids) and two identical **heavy (H) chains** (each usually containing about 440 amino acids). The four chains are held together by a combination of noncovalent and covalent (disulfide) bonds. The molecule is composed of two identical halves, each with the same antigen-binding site, and both L and H chains usually cooperate to form the antigen-binding surface (Figure 18–15).

The proteolytic enzymes papain and pepsin split antibody molecules into different characteristic fragments. *Papain* produces two separate and identical **Fab** (*f*ragment *a*ntigen *b*inding) **fragments**, each with one antigen-binding site, and one **Fc fragment** (so called because it readily crystallizes). *Pepsin*, on the other hand, produces one **F(ab′)$_2$ fragment**, so called because it consists of two covalently linked F(ab′) fragments (each slightly larger than a Fab fragment); the rest of the molecule is broken down into smaller fragments (Figure 18–16). Because F(ab′)$_2$ fragments are bivalent, they can still cross-link antigens and form precipitates, unlike the univalent Fab fragments. Neither of these fragments has the other biological properties of intact antibody molecules because they lack the tail (Fc) region that is responsible for these properties.

There Are Five Classes of H Chains, Each with Different Biological Properties[11,15]

In higher vertebrates there are five *classes* of antibodies, IgA, IgD, IgE, IgG, and IgM, each with its own class of H chain—α, δ, ε, γ, and μ, respectively; IgA molecules have α chains, IgG molecules have γ chains, and so on (Table 18–1). In

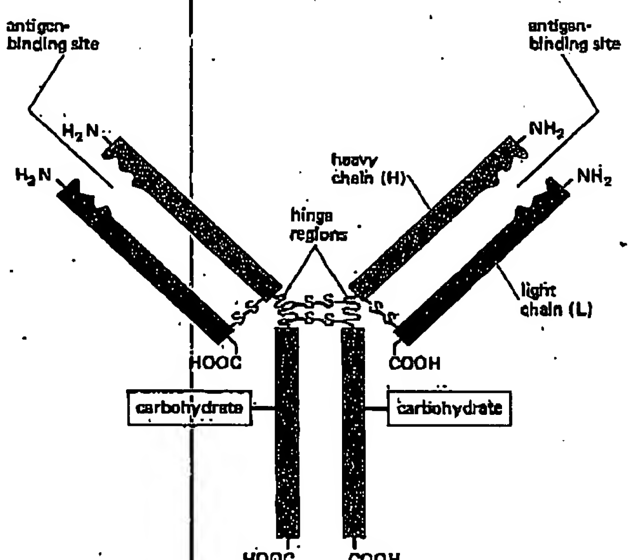

Figure 18–15 A typical antibody molecule is composed of two identical heavy (H) chains and two identical light (L) chains. Note that the antigen-binding sites are formed by a complex of the amino-terminal regions of both L and H chains, but the tail and hinge regions are formed by the H chains alone. Each H chain contains one or more oligosaccharide (carbohydrate) chains of unknown function.